Stage-Specific Embryonic Antigen-4 (SSEA-4) as a Distinguishing Marker between Eccrine and Apocrine Origin of Ducts of Sweat Glands

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TO THE EDITOR

Human skin has two major types of sweat glands: eccrine and apocrine. They differ in function, structure, and distribution over the surface of the body (Figure 1) (Sato et al., 1989). Although apocrine and eccrine secretory portions of sweat glands are clearly morphologically distinctive, their ducts are, to date, histologically and immunohistochemically indistinguishable. The ability to make this distinction is of a great significance, especially in the case of an axillary region, where both types of sweat glands are distributed close to each other. The origin of a particular duct can be identified only when its orifice is visible; in other words, an eccrine duct opens directly into the epidermis, whereas an apocrine duct opens into the infundibular portion of a hair follicle (Sato et al., 1989) (Figure 1a, 1b). In our previous study, we described stage-specific embryonic antigen-4 (SSEA-4) as a marker of ductal cells of eccrine sweat glands (Borowczyk-Michalowska et al., 2017). Here, we investigated whether SSEA-4 is present in ductal cells of apocrine sweat glands as well.

Skin biopsy samples were obtained from the axilla of nine healthy patients undergoing plastic surgery: five female patients aged 10, 31, 60, 74, and 80 years and four male patients aged 30, 31, 40, and 50 years. The written informed patient consent and approval of the ethics committee were obtained under Polish law (no. KBET/72/B/2008). Skin samples were fixed and stained as described by Borowczyk-Michalowska et al. (2017). The details of antibodies used are described in the Supplementary Materials and Methods online.

To address the question of whether SSEA-4 is also present in apocrine sweat glands, sections first were stained for carcinoembryonic antigen (CEA) to localize structures of both types of sweat glands (see Supplementary Figure S1a, S1b online). CEA expression is solely restricted to sweat glands in human skin, and it is present at the luminal surface of both eccrine and apocrine ductal cells, as well as in the eccrine secretory portion (Li et al., 2009; Saga, 2001). In the axillary eccrine sweat glands, as in our previous study, SSEA-4 was found to be detected in all cells along the full length of the duct, that is, in the intraepidermal acrosyringium and throughout the dermis to the coiled section leading to the secretory portion (Figure 1a). Subsequently, the luminal cells co-expressed both types of embryonic antigens (Figure 2a). We identified ducts of apocrine sweat glands as structures that were positive for CEA and localized within hair follicles or within the closest proximity of them (see Supplementary Figure S1a). The ductal cells of apocrine origin had no expression of SSEA-4, including in the upper part where duct opens directly into the hair canal and in the dermal portion leading to the secretory coil (Figures 1a and 2a). To further confirm our findings, we analyzed the expression of SSEA-4 concomitantly with several types of keratins. Although the presence of keratins is characteristic of all epithelial cells, they represent a complex family, with different proteins being expressed in specific types of cells. In eccrine ducts, keratin (K) 16 was detected in both layers of cells with luminal cells being more predominantly stained, whereas ductal cells of apocrine sweat glands expressed K16 uniformly (Figure 2b, Figure S1d). Both eccrine and apocrine ducts, on the other hand, showed a weak and variable reaction for K17, with only single cells being strongly stained (Figure 2b, Supplementary Figure S1d). In contrast to apocrine ductal cells, which did not express SSEA-4, in eccrine ducts expression of glycosphingolipid was always detected (Figure 2b). In rare instances, the single dots of SSEA-4 signal were visible in luminal cells of apocrine ducts; however, this signal was very weak and still clearly distinguishable from the one observed in the eccrine ducts (see Supplementary Figure S1d). Additionally, our results showed that no immunoreactivity for SSEA-4 was present in secretory cells of both eccrine and apocrine glands, although expression of K7, a marker of secretory coils, was clearly visible (Figure 2c). Thus, our findings provide compelling evidence supporting the notion that SSEA-4 may be the previously unreported marker that allows for distinction between eccrine and apocrine ducts of sweat glands.

In recent decades, several markers have been proposed to be characteristic of either eccrine or apocrine ductal cells. However, in practice, none of these markers have provided a reliable and reproducible way to make this distinction (Fuertes et al., 2013). Because CEA expression is detected in both eccrine and apocrine sweat glands, it is not a useful discriminative marker. K16 is expressed in skin, not only in ductal cells of both eccrine and apocrine origin, but also in basal cells of epidermis (Langbein et al., 2008). K17, on the other hand, is present mainly in myoepithelial cells of secretory coils but in hair follicles and sebaceous glands as well (Moll and Moll, 1992; Schön et al.,

Abbreviations: CEA, carcinoembryonic antigen; K, keratin; SSEA-4, stage-specific embryonic antigen-4

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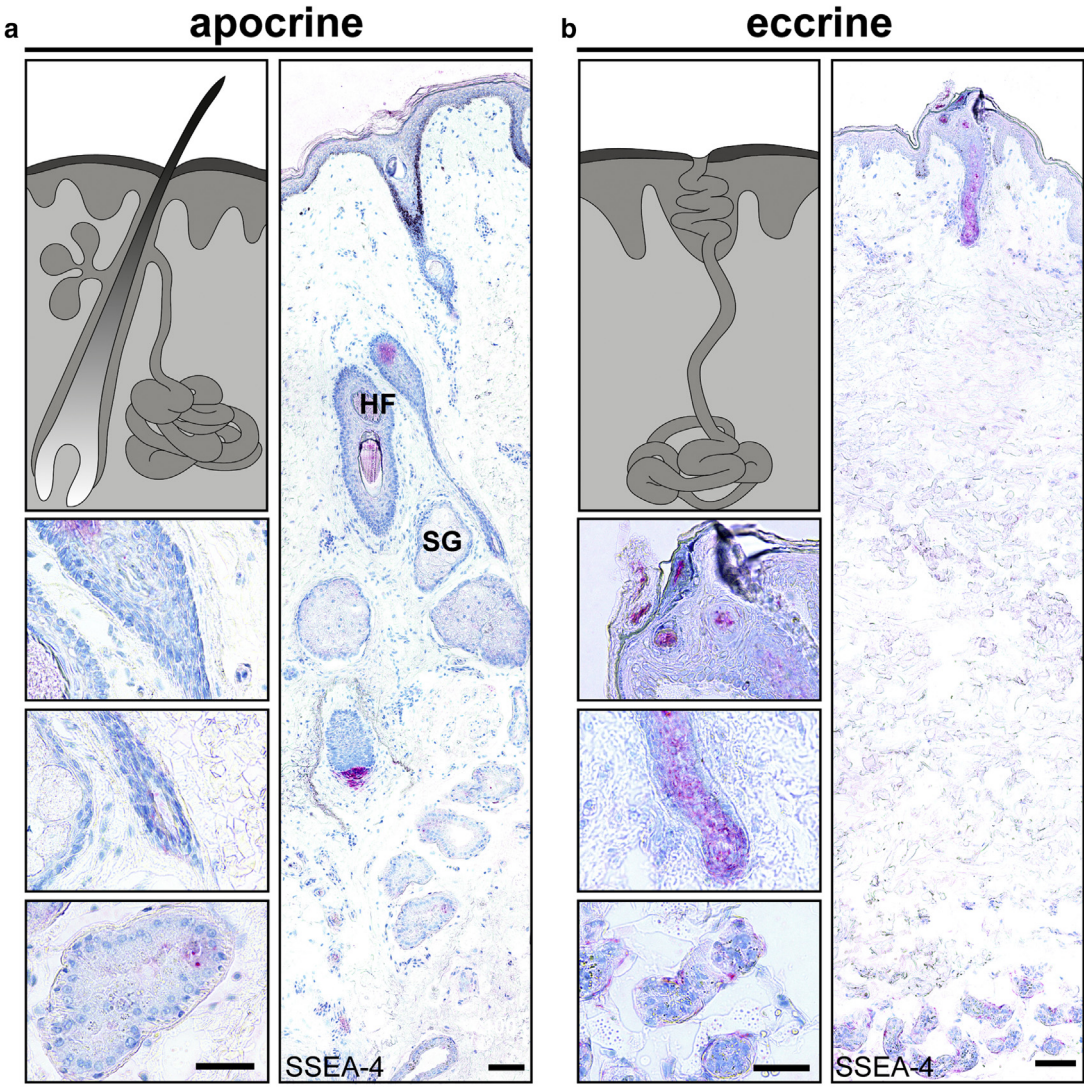

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	Function	Distribution	Sweat	Structure	Activation
Eccrine	Regulation of body temperature	Most body regions	Watery salt-based liquid, mainly sodium chloride, potassium and bicarbonate	*Opening*: Skin surface *Duct*: Long *Secretory coil*: Small lumen	Increase of body temperature (heat, exercise, fever, stress)
Apocrine	Not clear but potentially creates body odour	Only hairy body regions (axillae, perineum etc.)	Oily and viscous fluid rich in proteins, lipids and odoriferous substances	*Opening*: Hair follicle *Duct*: Short *Secretory coil*: Dilated lumen	Emotional stimuli (stress, excitement)

Figure 1. Expression of SSEA-4 antigen in human apocrine and eccrine sweat glands. Immunohistochemical detection of SSEA-4 antigen expression within (**a**) the entire apocrine and (**b**) eccrine sweat glands. Inserts show magnified views of respective parts of glands. Schematic illustrations of two types of sweat glands are included. (**c**) Major features of eccrine and apocrine sweat glands in humans. HF, hair follicle; SG, sebaceous gland; SSEA-4, stage-specific embryonic antigen-4. Scale bars = 100 μm (low magnification) and 50 μm (high magnification). The ImageJ Stitching Plugin was used for image reconstruction (Preibisch et al., 2009).

1999). Thus, the presence of K16 and K17 in the multiple epithelial cells does not make them reliable markers for apocrine-eccrine distinction. Another antigen frequently mentioned to be specific for the apocrine type of glands is GCDFP-15. However, as a component of fluid secreted by cells, it is solely present in the secretory portion and not in the excretory ducts of apocrine sweat glands (see Supplementary Figure S1c). Moreover, according to the literature, GCDFP-15 may also be detected in eccrine glands; thus, its application as a distinguishing marker is questionable (Viacava et al., 1998).

The ability to distinguish the origin of ductal cells is of particular interest for

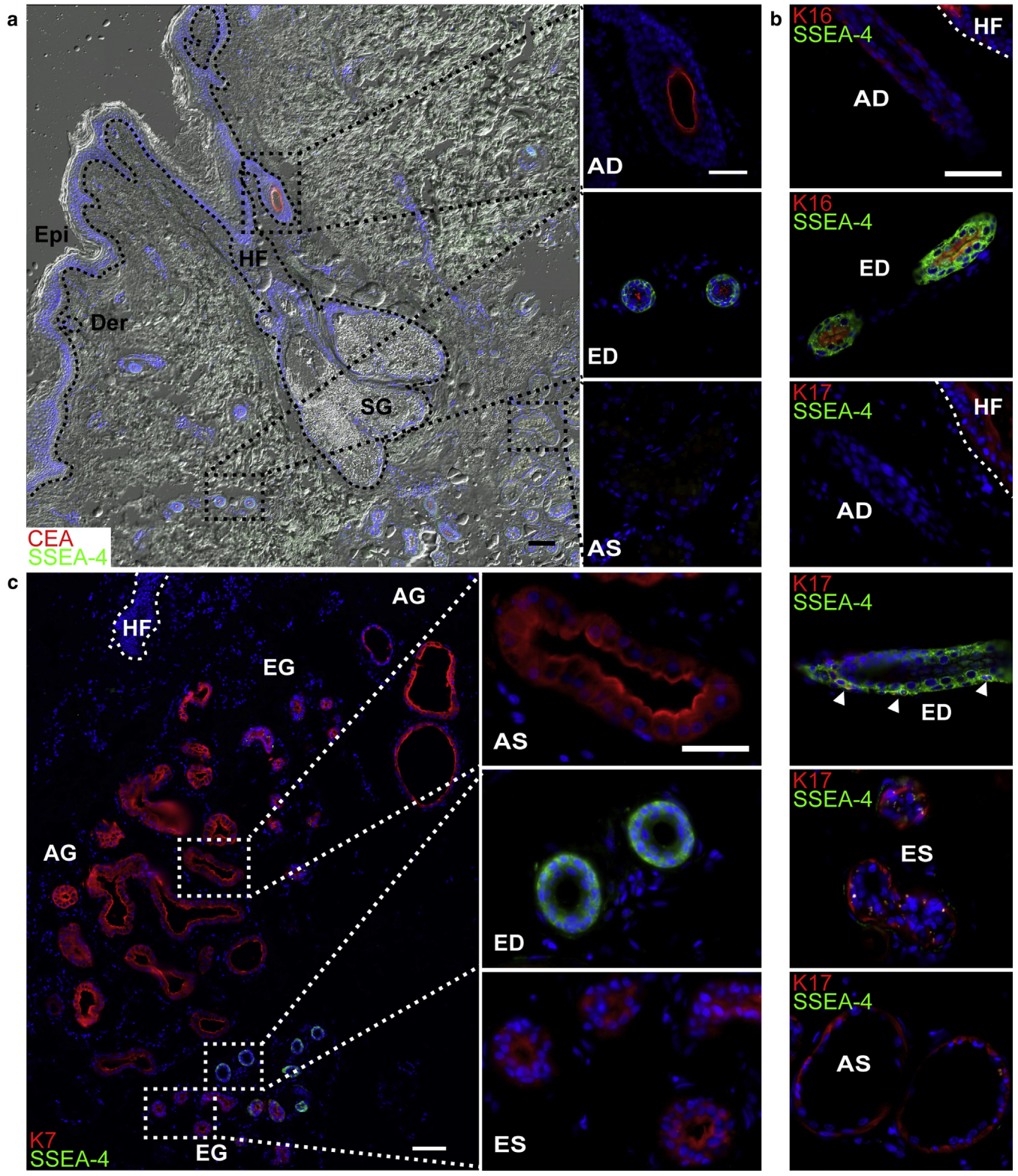

Figure 2. Expression patterns of SSEA-4 and sweat gland markers in human axillary skin. Double immunofluorescence detection of sweat gland markers CEA, K7, K16, K17, and SSEA-4 antigen in sweat glands. (**a**) Staining for SSEA-4 (green) and CEA (red); (**b**) staining for SSEA-4 (green) and K16 or K17 (red); (**c**) staining for SSEA-4 (green) and K7 (red). Nuclei (blue) were counterstained with Hoechst 33258. Inserts show magnified views. Arrowheads indicate reaction for K17. Scale bars = 100 μm (low magnification), 50 μm (high magnification). AD, apocrine duct; AG, apocrine sweat gland; AS, apocrine secretory portion; CEA, carcinoembryonic antigen; Der, dermis; ED, eccrine duct; EG, eccrine sweat gland; Epi, epidermis; ES, eccrine secretory portion; HF, hair follicle; K, keratin; SG, sebaceous gland; SSEA-4, stage-specific embryonic antigen-4.

the development of tumor diagnostic markers, because currently the combination of multiple antibodies is necessary to create a useful panel specific for apocrine and eccrine metastatic sweat gland carcinomas (Chintamani et al., 2003). In the light of these data and to the best of our knowledge, SSEA-4 is the first antigen enabling histological distinction between ducts of eccrine and apocrine sweat glands. If the expression profile of SSEA-4 is maintained during the malignant transformation of cells derived from the ducts of sweat glands, it can be assumed that it can be a very valuable diagnostic marker in the future.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2017.07.003.

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